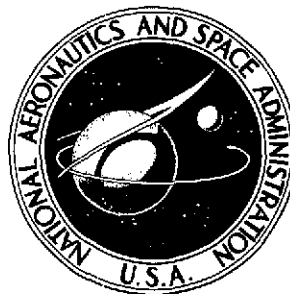


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ADENOSINE TRIPHOSPHATE (ATP) AS A POSSIBLE INDICATOR OF EXTRATERRESTRIAL BIOLOGY

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<p>Abstract</p> <p>The ubiquity of adenosine triphosphate (ATP) in terrestrial organisms provides the basis for proposing the assay of this vital metabolic intermediate for detecting extraterrestrial biological activity. If an organic carbon chemistry is present on the planets, the occurrence of ATP is possible either from biosynthetic or purely chemical reactions. However, ATP's relative complexity minimizes the probability of abiogenic synthesis.</p> <p>A sensitive technique for the quantitative detection of ATP has been developed using the firefly bioluminescent reaction. The procedure has been used successfully for the determination of the ATP content of soil and bacteria. This technique is also being investigated from the standpoint of its application in clinical medicine.</p>			
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ADENOSINE TRIPHOSPHATE (ATP) AS A POSSIBLE INDICATOR OF EXTRATERRESTRIAL BIOLOGY

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INTRODUCTION

Among the vast array of questions which man seeks to answer in his investigations of the upper atmosphere and outer space, none is more challenging and provocative than that pertaining to the existence of life outside terrestrial boundaries. Finding a satisfactory answer to this question is the purpose of intensive studies by several investigators.

Basic to these studies is the establishment of the parameters which define life. A definition amenable to operational manipulation is one which characterizes life as the capacity of a molecular complex to perform certain functional activities including metabolism, growth, and reproduction. If this definition is valid, it follows that a rational life-detection system must be capable of measuring phenomena related to these criteria.

To detect life is not difficult in many instances, especially in the presence of visible manifestations such as characteristic form, movement, or reproduction. It becomes increasingly difficult, however, as the size of the organism becomes microscopic; it becomes even more difficult as the number of organisms in a sample decreases. It is in the latter instances that classical life-detection techniques begin to depend upon one of the manifestations mentioned above: reproduction. Although adequate in many laboratory situations, detection techniques based on reproduction have the requirement of long time periods and rigorously controlled conditions. Another factor to be considered is that, in order to detect life beyond the surface of earth, techniques amenable to remote operation must be employed.

DETECTION OF THE METABOLIC INTERMEDIATE ATP

It is to satisfy these requirements that one begins to consider the detection of certain vital metabolic intermediates as a means of life detection. The metabolic intermediate would have to fulfill certain requirements; it would have to be ubiquitous and specific for living organisms, of sufficient complexity to render a spontaneous abiogenic synthesis unlikely, and amenable to sensitive detection. A compound that fulfills these requirements is adenosine triphosphate (ATP). The structure of ATP is shown in Figure 1.

ATP, the primary storage form of biochemical energy, is a prime example of a relatively complex organic molecule present on earth. It is so specifically and ubiquitously associated with biological reactions that its presence is accepted as a positive indication of the present or past existence of a terrestrial organism. This correlation provides one strong rationale for the

use of an assay for this compound as a means of detecting biological activity in extraterrestrial material.

This assay takes on additional significance as the result of the work of several investigators¹⁻⁴ who have demonstrated the abiogenic synthesis of a number of complex biological intermediates, including ATP, under conditions approximating those believed to be the primordial earth environment. These investigations suggest that ATP was a component of the earth's prebiotic chemistry. Therefore, its detection in an extraterrestrial sample (devoid of biological activity as confirmed by other detection techniques) could indicate an evolutionary chemistry similar to earth's, but not yet culminating in the emergence of life.

The likelihood of finding extraterrestrial ATP is dependent upon the fundamental requirement for its synthesis: carbon-based chemistry. Carbon in the form of CO₂ has been detected in the atmosphere of Mars⁵ and Venus,⁶ and the Jovian atmosphere contains abundant quantities of methane.⁷ Findings of compounds hydrolyzable to amino acids in lunar soil samples have also been reported.⁸ Examination of meteorites has revealed the presence of hydrocarbons and derivatives including amino acids.^{9,10} Of great significance are the recent radio telescope observations revealing the presence of an array of carbon compounds of low molecular weight in interstellar clouds.¹¹ (Interstellar clouds are the matter from which planets are formed.) These compounds include formaldehyde, hydrogen cyanide, ammonia, formic acid, formamide, and methanol; all are implicated in chemical and biological evolution on earth.

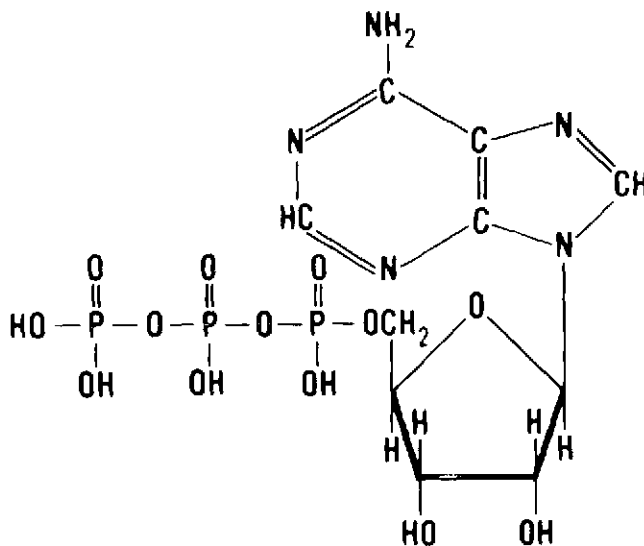


Figure 1. Chemical structure of adenosine triphosphate.

The occurrence of these intermediates in interstellar dust and meteorites throughout the galaxy strongly suggests that life anywhere in the galaxy would have a chemical makeup similar to that of terrestrial life and would quite likely involve ATP. It can be assumed that an extraterrestrial carbon-based chemistry would be subject to the same thermodynamic restraints that govern the permutations of terrestrial carbon compounds and thus, under similar environmental conditions, could follow a chemical evolutionary pathway leading to the synthesis of ATP. This assay will be of particular value in conjunction with life-detection experiments involving metabolism and growth, as described by Levin et al.¹² and Vishniac,¹³ and also in experiments involving analysis of organic compounds.

THE ANALYSES OF ATP

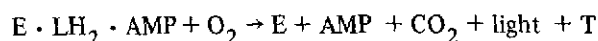
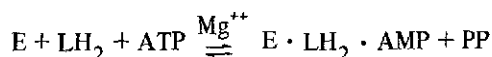
In the analyses of extraterrestrial material for ATP, at least four results are possible: (1) positive detection of ATP (along with positive proof of life by other detection systems), meaning that biological activity was present and resembled terrestrial life with respect to this compound; (2) positive indications of life in the absence of ATP, which point to life basically different from that found on earth; (3) detection of ATP in the absence of any life detected by other systems could mean that no life existed as detectable by the techniques employed and that the ATP was of abiogenic origin; and (4) the absence of ATP together with negative results from other life-detection systems, indicating either the absence of life or the presence of an exotic life form undetectable by the procedures used. However, in consideration of (3) above, our investigations with terrestrial soils show that, under certain conditions, ATP may remain stable for indefinite periods after it is released from a dead cell.¹⁴ Thus, detection of ATP in the absence of other positive determinations of life might mean that the compound was present as a result of abiogenic synthesis or as the trace of a life form long since dead. ATP detection could also mean that the chemical evolution had not yet reached the stage for the presence of life.

In order to realize the full potential of ATP as a monitor for the presence of life in space, it becomes necessary to select and develop a method with which it can be assayed with the highest degree of sensitivity, accuracy, and rapidity.

There are two general techniques by which ATP may be measured. The first technique requires its isolation in the pure state, after which it can be assayed by means of ultraviolet spectrophotometry.¹⁵ The second technique employs an enzyme system in which ATP is a substrate. The latter technique enjoys the advantage of not requiring that ATP be in a pure state, thus providing measurement procedures with simplicity and speed that cannot be approached by the first technique.

The choice of an enzyme system for ATP assay is dictated mainly by the degree of sensitivity of the assay methods for the products formed during the reaction between ATP and the enzyme. In most of the enzymatic assays of ATP at very low concentrations, the primary enzyme is coupled with one in which a pyridine nucleotide is reduced at a rate proportional to the ATP concentration. Reduced pyridine nucleotide, which has a very high extinction coefficient, is then assayed at 340 nm in the ultraviolet spectrophotometer.

Another enzymatic reaction requiring ATP, which appears to have far greater potential, is the bioluminescent reaction occurring in fireflies. The reaction mechanism has been well established by the excellent work of McElroy¹⁶ and his associates. In brief, the light emission in firefly bioluminescence results from the steps shown below.¹⁷



where E = firefly luciferase, LH_2 = reduced luciferin, ATP = adenosine triphosphate, AMP = adenosine monophosphate, PP = pyrophosphate, and T = thiazolinone.

The quantity of light emitted during the reaction is a function of the concentration of luciferase, luciferin, ATP, and O_2 . Therefore, in the presence of excess luciferase, oxygen, and luciferin, the total emitted light is a direct function of the concentration of ATP. It also has been shown that, under appropriate conditions, the peak light intensity is a linear function of the quantity of ATP, thus allowing the assay to be made in a much shorter period of time.¹⁸

The assay procedure that has been developed¹⁸ consists of injecting either standard ATP solutions or cellular extracts containing ATP into a cuvette containing the enzyme system (purified luciferase, synthetic luciferin, magnesium ion, and buffer).

Figure 2 is a schematic of a basic instrumentation system for performing the assay. The system consists of a rotary reaction chamber coupled to a photomultiplier tube. A section of the rotary chamber is cut out to accommodate a 6- by 50-mm glass cuvette. Immediately above the cuvette holder is a small injection port through which ATP is injected by needle and syringe into the enzyme solution. The signal from the photomultiplier tube is amplified, and the dc signal from the amplifier can be observed by a variety of means (recorder, oscilloscope, and so forth). Commercial instruments that are available for observing the dc signal include photometers made by American Instrument Co., E. I. DuPont De Nemours & Co., and JRB, Inc.

A concentration curve showing maximum intensity as a function of ATP concentration is shown in Figure 3. Although not shown, instrumentation of sufficient sensitivity has been developed by this laboratory to allow the detection of $1 \times 10^{-8} \mu\text{g}$ of ATP.¹⁹

Another obstacle of some magnitude that will confront any planetary-lander experiment is the effect of dry heat sterilization: a current NASA requirement. This is a difficult requirement for much of the hardware to meet and would seem impossible for an enzyme and necessary cofactors to meet. However, a technique has been developed in this laboratory that prevents a loss of biological activity by firefly luciferase and luciferin during heating.²⁰

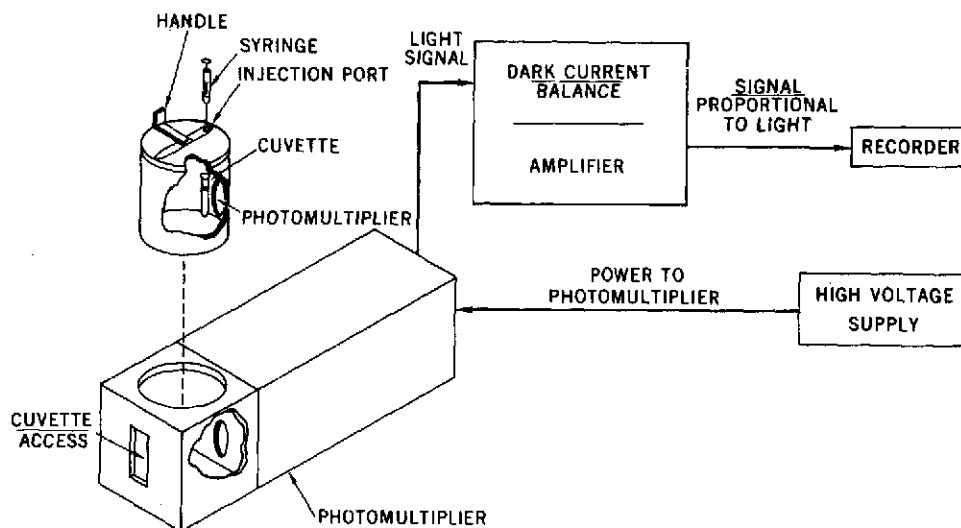


Figure 2. Light measuring instrumentation.

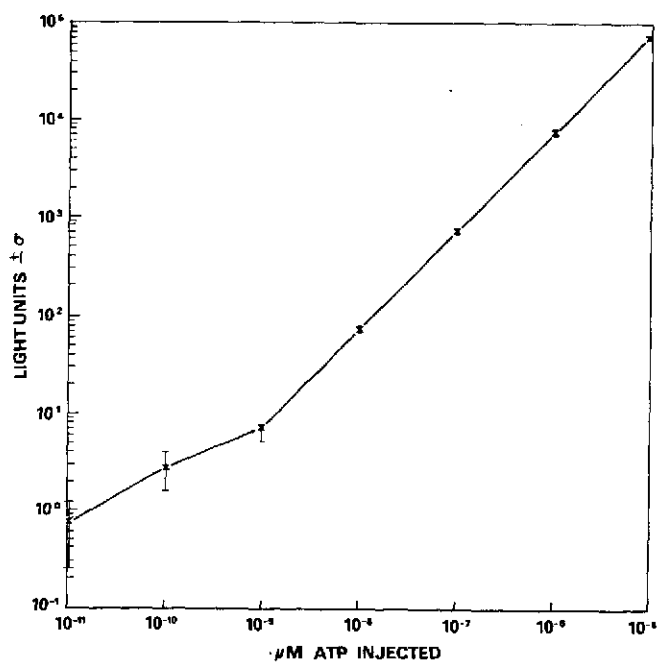


Figure 3. The initial peak height as a function of ATP concentration.

The necessary conditions include encapsulation in a molecular filtration gel, complete removal of H₂O by ultrahigh vacuum, and exposure to sterilization temperature in the complete absence of oxygen. Approximately 40 percent of the activity was retained after exposure of the enzyme system to a temperature of 408 K (135°C) for 36 hours.

The feasibility of the use of this assay for the sensitive quantitative detection of ATP in living organisms has been demonstrated with a variety of bacteria as shown in the following list. The amount of ATP per cell in the listed micro-organisms was determined by the firefly luciferase assay.

Organisms	μg ATP/cell (× 10 ⁻¹⁰)	Organisms	μg ATP/cell (× 10 ⁻¹⁰)
<i>Bacillus cereus</i>	1.10	<i>Micrococcus lysodeikticus</i>	1.30
<i>Bacillus coagulans</i>	1.70	<i>Mycobacterium phlei</i>	1.90
<i>Bacillus globigii</i>	5.40	<i>Mycobacterium smegmatis</i>	8.90
<i>Brevibacterium helvolum</i>	0.37	<i>Pseudomonas aeruginosa</i>	1.00
<i>Enterobacter aerogenes</i>	0.28	<i>Pseudomonas fluorescens</i>	3.10
<i>Erwinia carotovora</i>	0.44	<i>Proteus vulgaris</i>	1.80
<i>Escherichia coli</i>	1.00	<i>Sarcina lutea</i>	0.37
<i>Flavobacterium arborescens</i>	1.50	<i>Serratia marcescens</i>	1.00
<i>Gaffkya tetragena</i>	0.61	<i>Staphylococcus aureus</i>	0.64
<i>Klebsiella pneumoniae</i>	5.00		

In order to carry out this analysis, a technique for the quantitative extraction of ATP from bacterial cells was developed¹⁸ that involves *n*-butanol. As in situ biological investigations of other planets will most certainly use surface samples, studies designed to establish the necessary conditions for compatibility of the assay technique with soil samples have been conducted. The ATP content of a variety of soil types from many parts of the world has been measured. An excellent correlation between soil ATP and microbial count was obtained in most cases.¹⁴

While adequate instrumentation is available for the use of this technique on earth, extra-terrestrial investigations will require automated instrumentation that is capable of performing all of the assay steps on remote demand. Progress toward the realization of such instrumentation has been reported.²¹

A number of investigations at various stages of development are being carried out by us in collaboration with two hospital centers. The goals of these studies include the development of rapid automatable techniques for the detection of infection in biological fluids, rapid antibiotic susceptibility tests, viral detection techniques based on host-cell ATP changes, and techniques for monitoring kidney transplants. Progress made in the use of this technique for detection of bacteria in urine is exemplified in Table 1 which shows the correlation between colony count and count by ATP. The discrepancies between the luciferase method

and colony count are possibly due to various causes: trace amounts of nonbacterial ATP, the necessity of assuming an average value for ATP content per bacterium, the inability of some bacteria to produce colonies in the growth environment, and the presence of ATP within bacteria that are not dividing. Colony count technique requires 24 to 48 hours, while the count by the luciferase procedure requires 1 hour.

SUMMARY

In summary, the progress in instrumentation and analytical procedure allows us to view the ATP assay of extraterrestrial matter as a practical experiment. In concert with other exobiological experiments, the results from such an assay could provide insight into the biological status of other celestial bodies in our solar system.

An important ramification of the methodology developed for extraterrestrial ATP detection is its possible usefulness for a variety of applications on earth. As has been described, ATP is a constituent of all living organisms, thus making it an excellent monitor for micro-organisms such as bacteria and is also a sensitive indicator of the physiological state of any living cell.

Table 1

Bacterial Counts per ml from Clinical Urine Specimens Comparing the Luciferase Centrifugation Procedure with the Agar Pour Plate Method.

Specimen Number	Luciferase	Pour Plate
1	3.2×10^8	$> 10^7$
2	3.1×10^4	4×10^3
3	$< 10^3$	$< 10^2$
4	$< 10^3$	8×10^3
5	7.0×10^3	1×10^5
6	$< 10^3$	$< 10^2$
7	9.4×10^3	2×10^2
8	$< 10^3$	$< 10^2$
9	3.1×10^7	$> 10^7$
10	3.1×10^7	$> 10^7$
11	$< 10^3$	$< 10^2$
12	2.7×10^4	$< 10^2$
13	8.4×10^7	$> 10^7$
14	3.2×10^4	9×10^2
15	2.1×10^8	8×10^6
16	1.3×10^7	1×10^7
17	1.6×10^7	$> 10^7$
18	8.6×10^4	4×10^4
19	$< 10^3$	1×10^2
20	2.4×10^4	$< 10^2$
21	3.1×10^6	5×10^6
22	5.5×10^3	1×10^3

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 Greenbelt, Maryland May 21, 1973
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